A CYTOLOGICAL STUDY OF THE SPOROZOITES OF EIMERIA CAVIAE, A COCCIDIAN PARASITE OF THE DOMESTIC GUINEA PIG, CAVIA PORCELLUS

An abstract of a Thesis by
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The problem. The present study was undertaken in order to provide information concerning the morphological and cytological characteristics of the sporozoites of Eimeria caviae, Sheather (1924), a coccidian parasite of guinea pigs. Observations concerning the prepatent and patent periods, the symptomatology and pathology, and the morphological features of the oocysts of this species are also reported.

Procedure. Oocysts of Eimeria caviae, isolated from naturally infected guinea pigs, were excysted in vitro and the sporozoites studied in fresh and stained preparations. Fresh sporozoites were studied in the medium used for excystation, at room temperature, with the aid of a Zeiss RA and/or Zeiss photomicroscope equipped with achromatic objectives and bright field and phase contrast condensors. Smears for permanent preparations were made by smearing No. 1 cover slips with Meyer's adhesive and freshly excysted sporozoites. Sporozoites stained with the Giemsa method were fixed in methanol and those stained with Harris' hematoxylin and eosin were fixed in Zenker's fluid. Sporozoites stained with the periodic acid-Schiff (PAS) and the Feulgen methods were fixed in Bouin's fixative.

Findings. Freshly excysted sporozoites were motile and exhibited flexion, probing, gliding and rotational movements. The sporozoites were curved in shape with the anterior end tapering to a point and the posterior end being more rounded and blunt. All sporozoites had one anterior and one posterior refractile body with a nucleus between these bodies. Staining reactions revealed refractile bodies, numerous granules distributed within the cytoplasm and a nucleus containing peripherally distributed chromatin; no nucleolus was observed.

Sporulated oocysts were ellipsoidal and brownish-yellow in color. An oocyst residium was not observed. A cone-shaped Stieda body and membrane-bound sporocyst residium were present. The oocyst wall was smooth and consisted of two layers.

The prepatent period of Eimeria caviae was found to range from 11 to 13 days; the patent period ranged from 7 to 13 days. Symptoms of infection in guinea pigs experimentally infected with E. caviae were loss of appetite, constipation,

diarrhea, ruffled hair, and a "hunched" posture. Apathetic behavior was also a constant symptom.

Conclusion. The cytological observations of the sporozoite of Eimeria caviae in the present study agree closely with those observed previously for this and other species. The prepatent and patent periods, symptomatology and pathology, and morphological features of the oocysts also agree with that information previously reported for this species.

Recommendations. While information concerning the morphology of the sporozoites of Eimeria caviae is presented in the present study, many important problems concerning their mechanism of invasion of host cells, their excystation process, and the role they play in the immune response remain to be solved.

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INTRODUCTION

The present study provides information concerning the cytological characteristics of the sporozoites of Eimeria caviae, Sheather (1924), a coccidian parasite of the domestic guinea pig, Cavia porcellus. The sporozoite is important in that it is the stage in the coccidian life cycle which first invades the cells of the host, initiating infection. Also, it is often useful for specific diagnosis. It is hoped that this study will contribute to our understanding of the sporozoite stage of Eimeria caviae and perhaps of the coccidia in general. Eimeria caviae was chosen for this study because of the apparent lack of detailed information available about this species, and because of the ease with which guinea pigs could be obtained and reared in the laboratory.

REVIEW OF LITERATURE

Labbé (1899) was apparently the first to report coccidian oocysts in the intestinal contents of guinea pigs. He believed that the oocysts which he observed represented a variety of a coccidian species which had been previously reported from rabbits. He did not, however, discuss the parasite in detail. Strada and Traina (1900) described a coccidium from guinea pigs in Italy, which they called Coccodium oviforme, and reported that infections with this

coccidium were associated with diarrhea, emaciation and death. There was no discussion of sporozoites presented.

Bugge and Heinke (1921) found 73% of 180 guinea pigs from Germany to harbor coccidia and regarded the infection as common and widely distributed. They also noted that sporulation of E. caviae began in 2 to 3 days and was completed in about 5 to 8 days in boric acid or thymol at room temperature. These authors also reported the occurrence of diarrhea in guinea pigs infected with this parasite and a marked intestinal inflammation resulting from the infection. With reference to the sporozoite stage, Bugge and Heinke (1921) stated only that two, sickle-shaped, slightly curved sporozoites appeared within the sporocysts. However, the characteristics of these sporozoites were not discussed in detail. They also reported the developmental states of E. caviae to occur only in the upper portions of the colon.

Sheather (1924) described the life cycle of a coccidium which he isolated from a guinea pig purchased for experimental purposes. He identified this coccidium as a species of Eimeria, to which he gave the name Eimeria caviae. He stated that endogenous development appeared to take place only in the epithelium of the colon, especially in the upper portions with the frequency of endogenous development decreasing in the lower portions. Using 5% potassium dichromate solution at room temperature, he found that oocysts of E. caviae from different guinea pigs required

different times to complete sporulation, the range being from 5 to 8 days. Sheather (1924) found oocysts of E. caviae in the feces of infected guinea pigs as early as 7 days after inoculation and as late as 13 days after inoculation. When the infection was severe, almost every cell of the mucosa of the colon appeared to contain stages of the parasite. While discussing the schizonts, microgametocytes and macrogametocytes in some detail, Sheather (1924) mentioned sporozoites only as they related to the process of sporulation. Andrews (1930) reported that oocysts from guinea pigs all excysted in the small intestine, but failed to discuss sporozoites.

Henry (1932) using the method of Tyzzer (1929) noted that sporulation of E. caviae oocysts began within the twenty-four hour period following the onset of incubation in 2% potassium dichromate. Sporulation was complete in 2 to 3 days. Henry (1932) also noted that oocysts of E. caviae sporulated quite readily in normal salt solution. She also noted the prepatent period of E. caviae in guinea pigs previously uninfected with this species to be 11 to 12 days. Henry (1932) stated that in all but one of the experimentally infected animals studied, diarrhea was a constant symptom. She also noted that after the first indications of diarrhea were present, an occasional formed stool may be passed and that on the day following the appearance of marked diarrhea, only diarrheic stools occurred

with an increase in the quantity of fecal material being discharged. Diarrhea usually continued until the death of the guinea pig, but occasionally toward the end of the life of the animal, a string of very small pellets was passed. Constipation was a common symptom, and this symptom was most often noticed on the eleventh or twelfth day, but rarely later. At about the time of the occurrence of diarrhea, guinea pigs became visibly ill, remaining very quiet with hair ruffled and with the body contracted in such a manner that the posterior portion of the animal was noticeably drawn under the rest of the body. A very marked loss of appetite was observed by Henry in all infected animals. In discussing the pathology of this species, Henry (1932) found lesions of coccidiosis along with numerous oocysts of Eimeria caviae in the large intestine of 24 guinea pigs that apparently died of coccidiosis. In all cases, the lungs, liver and spleen were normal in appearance and no changes were noticed in the small intestine or in the stomach, although a few isolated lesions of coccidiosis were seen in the small intestine. In the colon, endogenous stages were found in the epithelial cells lining the crypts of Lieberkuhn. In many places, the entire mucosa had been destroyed, leaving large spaces containing some cellular debris and oocysts. Ruptured capillaries, with resulting hemorrhage, were observed in several cases. Henry's only mention of the sporozoites of E. caviae was the

possible role they might play in the cellular response of the host to infection, and that during sporulation, each of the four sporoblasts were gradually transformed into sporocysts containing two sporozoites and some rather granular residual material. Lapage (1937) found E. caviae in two laboratory groups of guinea pigs. In one population of 24 guinea pigs, 40% were infected while in a second population of 57 guinea pigs, 91% were infected. Lapage discusses variations in different phases of the life history of Eimeria caviae as well as the pathology of the parasite. He noted that this parasite produced little host tissue reaction and in general affected the host surprisingly little. The only mention of sporozoites by Lapage was that they did form in 60-70% of the oocysts which were allowed to incubate for 11 days at laboratory temperatures.

Although these earlier studies provided information relative to the life cycle, pathology and prevalence of E. caviae, detailed information concerning the cytology and general structure of the sporozoites of this organism were lacking. Levine and Ivens (1965), in their recent monographic review of the coccidian parasites of rodents, made no reference to the cytological characteristics of the sporozoites of this species, nor did Kheysin (1972) in his comprehensive review of the biology of the coccidia. However, some information about the cytological and structural aspects of the sporozoites of other Eimeria species as

inferred from histochemical and light microscopy studies is available. Smetana (1933) noted that sporozoites of Eimeria stiedae from the rabbit were crescent-shaped and pointed at the anterior end, and that the middle and posterior portions contained large, homogeneous, fluid-like droplets, now known as refractile bodies. Numerous small refractile granules were also seen in various locations throughout the sporozoites.

Using brightfied and phase-contrast microscopy, Nyberg and Hammond (1965) described the living sporozoites of 4 species of Eimeria: E. bovis, E. auburnensis, E. ellipsoidalis and E. zurnii. The excysted sporozoites of E. bovis were found to agree with a description given earlier by Hammond, Bowman, Davis and Simms (1946) except that the sporozoites were slightly longer (12 to 15μ as compared with 13.5 to 15.5μ). Also, Nyberg and Hammond (1965) observed a projecting tip at the anterior end of the sporozoites of E. bovis which had not been previously reported. The nucleus appeared vesicular with a small, somewhat eccentric nucleolus. Hammond, Chobotar and Ernst (1968), who studied the cytological aspects of the sporozoites of E. bovis, E. auburnensis and an Eimeria species from the Ord Kangaroo Rat noted that the refractile bodies in sporozoites of E. bovis stained deeply with iron hematoxylin and bromphenol-blue but were pink in hematoxylin and eosin and in Giemsa preparations. The refractile bodies also appeared

orange with the modified periodic acid-Schiff-analine orange (PAS-AO) method and reacted negatively to the Feulgen and protargol methods. In preparations stained with Sudan black B, the refractile bodies stained a light gray. In Feulgen preparations of sporozoites, a positive reaction was seen only at the periphery of the nucleus, showing a marginal layer of chromatin with inconspicuous clumps which occurred at irregular intervals along this layer. The nuclei in Giemsa preparations were usually obscured by numerous surrounding granules. In specimens prepared with the periodic acid-Schiff (PAS) method, many PAS-positive granules were observed. After 1 hour of diastase digestion, the reaction was negative, indicating that these granules were glycogen (Hammond et al., 1968).

In the same study by Hammond et al., (1968), the sporozoites of E. auburnensis differed in some respects from those of E. bovis. The anterior portion of the body was more changeable in shape, undergoing greater elongation and retraction. Nyberg and Hammond (1965) found that the sporozoites of E. auburnensis were larger than those of E. bovis but like E. bovis contained 2 refractile bodies. Hammond et al. (1968) found an additional small spherical structure resembling a refractile body located at the extreme posterior end of the sporozoites of E. auburnensis. The reactions of the sporozoites of E. auburnensis with the various staining methods did not differ appreciably from

those of E. bovis (Hammond et al., 1968).

Nyberg and Hammond (1965) reported that free sporozoites of E. ellipsoidalis resembled those of E. bovis but the anterior end was more smoothly tapered, with the body remaining narrow for a greater distance from the anterior end than in the sporozoites of E. bovis. In Giemsa preparations and in the majority of free, actively moving sporozoites (Nyberg and Hammond, 1965), two refractile bodies of similar size were present, one near the posterior end and one near the anterior end. These bodies were smaller in proportion to the size of the sporozoite than in E. auburnensis. The nucleus was located centrally between the two refractile bodies, and there was an area of small granules immediately surrounding the nucleus.

Sporozoites of E. zurnii had a relatively large and prominent nucleus and a single relatively small refractile body (Nyberg and Hammond, 1965). In contrast, the sporozoites of E. bovis, E. auburnensis and E. ellipsoidalis had a relatively small nucleus and two more or less prominent refractile bodies. In the Eimeria species isolated from the Ord Kangaroo Rat (Hammond et al., 1968) the sporozoites resembled those of E. bovis in general appearance, but had a much larger posterior refractile body. Also, this species had much less glycogen than E. bovis and E. auburnensis. In PAS preparations, some specimens showed no positively reacting granules; others had only 1 to 5 of these which

were located between the refractile bodies. The nuclei of this species were similar in morphology to those of E. bovis and E. auburnensis in that a nucleolus was observed in each and the chromatin was located peripherally; some nuclei appeared as clumps. The sporozoites of E. acervulina were also reported to have nuclei of this kind (Pattillo and Becker, 1955). A nipple-like projection at the anterior extremity was also observed in the species from the Ord Kangaroo Rat (Hammond et al., 1968). While the present work and that of the authors cited herein was conducted with the light microscope, much excellent microscopy has been carried out on the ultrastructure of Eimeria species (see Hammond, 1973, for a review).

MATERIALS AND METHODS

The original inoculum used in the present study was obtained from 2 naturally infected guinea pigs housed in the pet department of a large department store in Des Moines, Iowa. Three hundred sixteen fecal samples from guinea pigs in pet stores in the Iowa cities of Winterset, Newton, Bettendorf, Davenport, Cedar Rapids and Des Moines were examined before these 2 infected animals were found. Droppings from each guinea pig sampled were collected either from a pan placed beneath each cage or directly from the bedding. The droppings from each cage were placed in separate baby food jars, each containing approximately 30 ml

of a 2% aqueous solution of potassium dichromate. These samples were placed in an ice chest and transferred to the laboratory. In some instances the potassium dichromate solution was added to the sample jars after the samples were returned to the laboratory. In the laboratory, each sample was recorded as to location and date of collection. All samples were comminuted with a wooden tongue depressor while still in the sampling jars. A 2 to 3 ml. aliquot of the mixed sample was then transferred to a 15 ml conical centrifuge tube into which 5 to 7 mls of Sheather's solution was stirred. The entire mixture was then centrifuged at 2,000 rpm for 7 minutes, allowing the oocysts to float to the surface of the Sheather's solution. After centifugation, material from the surface of the Sheather's solution was transferred with the aid of a flamed inoculating loop to a microscope slide, covered with a #1 cover slip which had been ringed with petroleum jelly, and observed under the light microscope for the presence of oocysts. Those samples found to be negative were discarded; those 2 found to be positive were allowed to sporulate and were used as the initial source of oocysts.

To facilitate sporulation, oocysts from the comminuted samples which were positive were concentrated by sedimentation in water for 24 hours, the supernatent was removed, and then the sediment was resuspended in a 2.5% aqueous solution of potassium dichromate. The resuspended samples were layered (5 mm deep) in loosely-covered, glass

Petri dishes and the oocysts allowed to sporulate at room temperature (18-22°C) for 15 days. After sporulation was completed, the oocysts were again concentrated by sedimentation, the supernatent poured off, and the sediment resuspended in fresh potassium dichromate. This suspension was then distributed in thin layers (2 to 3 mm deep) in each of several inverted plastic Petri dish lids into which equal amounts of Sheather's solution was mixed. Petri dish bottoms were carefully floated on this mixture, allowing the oocysts to rise and adhere to the underside of the Petri dish bottoms. The bottoms were carefully lifted away from the surface and the adhering oocysts washed into a clear beaker with jets of water from a wash bottle. The oocyst suspension was then washed by repeated sedimentation in water to remove any residual sugar and potassium dichromate. This suspension was then resuspended in buffered Ringer's solution and stored in the refrigerator at 5°C as a stock suspension for use as inoculum.

In order to determine the prepatent and patent periods, and in order to obtain a larger quantity of oocysts for inoculum, 6 guinea pigs obtained from Diamond Laboratories in Des Moines, Iowa, were inoculated with sporulated oocysts of Eimeria caviae obtained from the two naturally infected guinea pigs described above. Six days prior to inoculation, fecal samples from these 6 guinea pigs were examined daily to determine whether or not oocysts were

present. When all 6 were determined as being negative for coccidiosis, 3 were inoculated with 3,000 sporulated oocysts of Eimeria caviae and 3 with 24,000 sporulated oocysts. Oocysts were introduced, per os, with a 1 cc syringe equipped with a flexible polyethylene cannula. These guinea pigs, which ranged from two months to two years in age, appeared strong, sleek and healthy. They were housed in separate wire cages having wire bottoms, and were given fresh food, water and bedding daily. Bedding consisted of commercially prepared wood chips. Each animal was given one cup of nonmedicated, nonsterile, Purina Laboratory Animal Chow and generous amounts of lettuce daily. Fecal pellets collected daily from each inoculated animal were placed in 33 ml of a 2% aqueous solution of potassium dichromate. The samples were examined for the presence of oocysts as previously mentioned. Oocysts from positive samples were cleaned, concentrated, and sporulated as described previously, and stored at 5°C in Ringer's solution until needed for further study.

For excystation purposes, these stock suspensions of oocysts from experimentally infected guinea pigs were further cleaned by adding portions of the stock suspensions to 5 to 7 mls of 33% Sheather's solution and by centrifuging the mixture at 2,000 rpm for 5 minutes. After centrifugation, the heavier debris was distributed throughout the Sheather's solution in the lower portion of the tube while

the oocysts were suspended at the Sheather's-Ringer's interface. The Ringer's supernatant was carefully withdrawn by a Pasteur pipette and discarded. The layer of cleaned oocysts was removed carefully with a Pasteur pipette and transferred to 5 mls of distilled water in a clean 15 ml conical centrifuge tube. This suspension was washed repeatedly (2,000 rpm for 5 minutes) to remove any residual Sheather's solution. During the final rinse, the cleaned oocysts were concentrated in a small pellet at the bottom of the tube. Oocysts from this pellet were transferred to a tissue grinder containing a small amount of Ringer's solution and broken mechanically by grinding for 6 minutes. The entire contents of the tissue grinder were then transferred to each of two 15 ml conical centrifuge tubes. The tissue grinder was rinsed into these centrifuge tubes with about 1.0 ml Ringer's solution. The sporocyst-oocyst mixture was then centrifuged at 1800 rpm for 5 minutes and allowed to stand for 10 minutes (Doran and Farr, 1962). The supernatant was carefully decanted, 2 mls of excystation medium were added, and the tubes were agitated. These were then incubated in a water bath at 39°C for 2 hours. At the end of the 2 hour period, the sporocyst-sporozoite suspension was washed in Ringer's solution by repeated centrifugation (2,500 rpm for 5 minutes), the supernatant was removed, and the sporozoites in the pellet were ready for study.

The excystation medium, mentioned above, was similar to that used by Doran and Farr (1962). In the present study, 1 ml of fresh guinea pig bile, .25% trypsin and 10 ml of mammalian Ringer's solution was used. Fresh bile, obtained from the gall bladder of killed guinea pigs, was transferred directly to the Ringer's-trypsin mixture and stirred with a magnetic stirrer for 5 minutes. Excess bile was injected into sterile serum vials and frozen for future use. In guinea pigs maintained on a normal diet, only about 0.2 to 0.5 ml of bile was present in the gall bladder. In order to increase bile production, guinea pigs were maintained on a low fat diet of lettuce only, which increased bile production to as much as 1.8 to 4.5 mls. Those guinea pigs used for bile production were not kept in the laboratory, but were obtained directly from Diamond Laboratories as bile supply dictated.

Free sporozoites, obtained as indicated above, were studied in fresh and stained preparations. Fresh sporozoites were studied in the medium used for excystation, at room temperature, with the aid of a Zeiss RA and/or Zeiss photomicroscope equipped with achromatic objectives and bright field and phase contrast condensors. All measurements were made with the aid of an ocular micrometer while using bright field illumination and are herein expressed in microns unless otherwise indicated; range measurements are placed in parentheses following the means. Drawings are

made to scale from photographs and from direct observation.

Smears for permanent preparations were made by smearing a No. 1, 22 mm square, cover slip with Meyer's adhesive. The cover slip was placed adhesive-side up on a glass slide with the edge of the cover slip projecting slightly beyond the slide so that it could easily be grasped with forceps. The adhesive was allowed to dry to a slightly "sticky" film, (about 5 minutes, depending on humidity). With a Pasteur pipette, 2 to 3 drops of sporozoite suspension were placed in the center of the cover slip, after which it spread over the entire surface. The liquid was then allowed to evaporate slightly, bringing the sporozoites into contact with the adhesive. The liquid was not allowed to completely dry (the edges were dry, but the center still remained moist). This evaporating process takes from 5 to 15 minutes and must be carefully watched lest the preparation become destroyed by overdrying. The cover slips were then carefully placed, specimen-side up, in a Petri dish filled with the appropriate fixative. After fixation, the cover slips were removed, stained and mounted.

Sporozoites stained with the Giemsa method were fixed in methanol. Sporozoites fixed in Zenker's fluid were stained with Harris' hematoxylin and eosin. Sporozoites stained with the periodic acid-Schiff (PAS) method and the Feulgen method were fixed in Bouin's fixative. To determine whether PAS-positive granules contained glycogen, control

smears were treated with 1% diastase in 0.8% saline solution at 37°C for 1 hour. All permanent preparations were mounted in a synthetic mounting medium, and were photographed, drawn, and measured as indicated above.

RESULTS

Sporulation and Prepatent and Patent Periods

In the present study, 70% of the oocysts completed sporulation in 9 to 11 days at room temperature (19 to 22° c). Oocysts of Eimeria caviae were first seen in the feces of experimentally infected guinea pigs on the eleventh day after inoculation. Oocysts were never detected later than 25 days post-inoculation. Oocyst production was heaviest on day 14 post-inoculation. As indicated in Table 1, the prepatent period averaged 12 (11 to 13) days and the patent period 10 (7 to 13) days.

Clinical Signs

Guinea pig No. 1, inoculated with 3,000 sporulated oocysts, first showed signs of constipation 17 days post-inoculation. Loss of appetite was also noted at this time but no diarrhea was observed. Constipation continued through day 21, at which time appetite and stools returned to normal.

Guinea pig No. 2, having been inoculated with 3,000 sporulated oocysts, showed the first signs of constipation

Table 1. Prepatent and Patent Periods of Eimeria caviae (in Days)

Guinea pig No.	1	2	3	4	5	6
Prepatent Period	12	12	13	12	12	11
Patent Period	11	12	8	13	7	9

13 days post-inoculation; constipation continued through day 24. Sixteen days post-inoculation, the animal became visibly ill, grooming ceased, and the hair appeared ruffled and in general disarray. Control guinea pigs constantly groomed themselves and did not show this "unkempt" appearance. Loss of appetite was noticed 18 days post-inoculation. Twenty days post-inoculation, normal grooming behavior returned, and the animal appeared, in general, more mobile.

Constipation was first observed 14 days post-inoculation in guinea pig No. 3, which had been inoculated with 3,000 sporulated oocysts, and continued until 17 days post-inoculation at which time normal stools were passed. Loss of appetite occurred one day after the appearance of constipation. Apathetic behavior was first noticed on day 17, 3 days after the appearance of constipation. Stools were normal on day 18 and no oocysts were present. One day later, the guinea pig appeared to be recovering from its apathetic condition and appeared to return to normal by day 28.

Guinea pigs 4, 5 and 6 were inoculated with 24,000 sporulated oocysts each. Guinea pig 4 showed mild diarrhea 13 days after inoculation. About this time, this guinea pig confined itself to the corner of the cage and assumed a "hunched" position in which the hair appeared to ruffle. Control guinea pigs moved constantly and seldom assumed a

"hunched" position. By day 15, the guinea pig became anorexic and seldom moved. Stools and appetite returned to normal on day 18, at which time the animal's general behavior began to improve. Guinea pig 5 showed signs of constipation 12 days after inoculation. Food and water intake decreased progressively from day 13 to day 15. On day 13, normal colored stools consisting of 4 to 7 pellets connected together in 6 cm lengths were noticed and by day 15, severe diarrhea occurred. Although severe, the diarrhea lasted only 2 days and the animal returned to normal by day 18.

Stools having 4 to 7 connected pellets were also evident in guinea pig 6, 15 days after inoculation. Unlike animal No. 5, these stools became diarrheic within about 12 hours, and lasted 3 days. A "hunched" posture was noticed in this guinea pig also. Between day 19 and day 28, stools of connected pellets were again passed. The animal never seemed to fully return to normal in that stools became inconsistent and eating habits sporadic. The animal died two weeks later. No oocysts were observed in fecal samples taken from the animal's cage the day it died. All other experimental animals continued to live.

Six control guinea pigs observed during the studies of the experimental animals showed none of the symptoms present in infected guinea pigs. Control animals were always active and their appetites seemingly unsatiated as

they constantly nibbled either lettuce or pellets. Coat appearance also served as an excellent comparison between control and experimental animals as controls had slick, well-groomed coats while the experimental animals had coats which generally appeared "unkempt".

Excystation of Sporozoites

Excystation appeared to begin with a swelling of the Stieda body which protruded more than normal, then became indistinct and suddenly disappeared (Figures 11 and 12). Sporozoites were seen to move within the sporocysts before any noticeable change in the Stieda body occurred. In E. utahensis, it was noted that the Stieda body suddenly popped out (Hammond, Ernst and Chobotar, 1970). However, the present author's data can only note that the Stieda body suddenly disappeared; whether it popped out, was shot out, exploded out, etc., would only be mere conjecture and unsupported by accurate observations. As soon as the Stieda body became detached, one of the two sporozoites contained in the sporocyst inserted its pointed end into the opening created by the loss of the Stieda body and began to emerge (Figure 12). The earliest escape of sporozoites was observed 10 to 15 minutes after beginning of incubation and 90% had excysted after 1 hour. The conoid region appeared to be that portion of the sporozoite which emerged first (Figure 12); the rest of the sporozoite followed (Figure 13).

The body of the sporozoite was constricted to about three-fourths of its normal diameter as it passed through the small opening in the sporocyst wall. When the first sporozoite completed its process of emergence, which lasted about 20 to 30 seconds, the second sporozoite began to emerge in a manner characteristic of that of the first sporozoite. The freed sporozoites had the characteristic shape and appearance of Eimeria caviae sporozoites (Figures 14 and 15). With both sporozoites having emerged, an empty sporocyst with sporocyst residium remained (Figure 16).

Observation of Living Sporozoites

With bright field illumination, living sporozoites appeared banana-shaped with the anterior end tapering to a point and the posterior end appearing rounded and blunt (Figure 1). Both ends appeared more opaque than the central one-third of the sporozoite, which appeared translucent. Fifty extended sporozoites averaged 12.9 (8.1 to 18.9) in length by 2.6 (1.6 to 2.7) in width in the region of the anterior refractile body and 2.8 (2.1 to 3.7) in width in the region of the posterior refractile body (Figures 1 and 14). Fifty flexed sporozoites averaged 10.3 (8.1 to 15.6) in length (Figure 15). In extended specimens, posterior refractile bodies averaged 5.5 (2.7 to 8.1) in length by 2.9 (2.1 to 3.7) in width; these were located an average of 1.2 (1.6 to 3.7) from the posterior end. Fifty

anterior refractile bodies averaged 2.6 (1.6 to 4.8) in length by 2.6 (1.6 to 3.7) in width. Anterior refractile bodies were located about 2.8 (1.6 to 4.8) from the anterior end in extended specimens (Figure 1). The nucleus was located about 1.0 to 1.5 from the anterior end in extended sporozoites (Figures 1 and 7). Several granules of varying sizes were seen throughout the sporozoites with the greatest concentration observed in the middle and posterior regions. No vacuoles of any kind were observed.

Freshly excysted sporozoites were motile and exhibited flexing, rotating, probing, and gliding movements similar to those observed in Eimeria bovis by Hammond et al. (1968). Flexion occurred about every 2 minutes and involved the slow bending of the anterior one-third of the sporozoite toward the concave side of the body. Flexion continued until the anterior one-third of the body was directed posteriorly, changing the shape of the sporozoite from that of a crescent to that of a J-shaped configuration. Extension was rapid and often followed by gliding.

Gliding movements occurred periodically, and apparently randomly, with no set time interval between glides. Typically, the sporozoites were observed to glide for about 2 seconds, covering a distance ranging from 4 to 230 microns. Gliding always began from the extended position, never from the flexed. On a few occasions, rotation of the sporozoite was observed in which the sporozoites glided forward in

spiral fashion. The direction of rotation, whether clockwise or counterclockwise, was not determined because of the rapidity of movement. Probing in which the anterior end of the sporozoite moved laterally in all directions while the posterior end remained fixed, was also observed.

Living sporozoites remained active for about 5 hours in excystation medium at room temperature (18 to 22°C). After 4 additional hours at refrigerator temperature (5°C), some sporozoites still exhibited gliding, flexing and probing movements, but at a much slower rate than normal. After about 5 hours of refrigeration, sporozoites began to undergo degeneration in which 1 to 6 globule or blister-like protrusions appeared on the cell surface. The size and number of these protrusions increased with time of degeneration until the sporozoite seemed to disintegrate. At about the time the globule-like protrusions appeared, the cytoplasm became more granular. This too increased with the passage of time.

Observation of Fixed and Stained Sporozoites

Twenty-five sporozoites fixed in methanol and stained with Giemsa's stain averaged 12.7 (7.9 to 8.1) in length by 2.4 (1.4 to 2.8) in width in the region of the anterior refractile body, and 2.6 (1.6 to 2.7) in the region of the posterior refractile body. Refractile bodies were not measured in these specimens. In the area surrounding the

nucleus, 10 to 15 dark red granules ranging from 0.1 to 0.5 in diameter were observed (Figures 2 and 5). The nucleus itself was obscured by these red granules which surrounded it. The distribution of these granules was more concentrated anterior to the nucleus but several granules were present posterior to the nucleus. At the anterior end of the sporozoite, in front of the anterior refractile body, 5 to 12 dark red granules ranging from 0.1 to 0.5 in diameter were also present (Figures 2 and 5) and at the posterior end of the sporozoite, behind the posterior refractile body, 3 to 6 dark red granules ranging from 0.1 to 0.5 in diameter were observed; these latter granules were distributed between the posterior refractile body and the outer membrane of the sporozoite. The cytoplasm in the middle region of the sporozoites stained light blue and appeared homogeneous, while the anterior and posterior ends stained darker blue, appearing homogeneous. The refractile bodies stained very light pink. In several Giemsa stained preparations, as well as in some living preparations, a small nipple-like structure was observed at the anterior end of the sporozoite (Figure 14) and may represent the conoid.

In specimens fixed in Bouin's fluid and stained with the periodic acid-Schiff (PAS) procedure, numerous small PAS-positive granules were observed (Figures 3 and 6). After 1 hour of diastase digestion with control slides, corresponding granules were not observed, indicating that

the granules contained glycogen (Hammond et al., 1968) or amylopectin (Ryley, 1969). Numerous PAS-positive granules were concentrated in the middle of the sporozoite totally obscuring the nucleus. A single layer of PAS-positive granules was concentrated around both the anterior and posterior refractile bodies. Both the granules in the center of the sporozoite, in the region of the nucleus, and those surrounding the refractile bodies averaged about 0.3 (0.1 to 0.6) in diameter. Twenty-five sporozoites, stained by the PAS method, averaged 12.2 (7.6 to 16.4) in length by 2.2 (1.4 to 3.0) in width in the region of the anterior refractile body and 2.7 (2.0 to 3.6) in width in the region of the posterior refractile body. Although unstained, the refractile bodies were clearly outlined by the PAS-positive granules (Figures 3 and 6). The nucleus was not observed in these preparations.

Specimens fixed in Bouin's and Zenker's fixative and stained by the method of Feulgen and counter-stained with fast green, showed a thin peripheral layer of chromatin having 3 to 5 chromatin clumps lying at irregular intervals along this layer (Figures 4 and 7). No nucleolus was observed. The chromatin was distributed in an area of about 1.0 to 1.5 in diameter. In Feulgen preparations, 25 posterior refractile bodies averaged 5.3 (2.5 to 7.9) in length by 2.8 (2.1 to 3.6) in width. Twenty-five anterior refractile bodies averaged 2.5 (1.6 to 4.7) in length and

2.6 (1.6 to 3.7) in width. A large percentage of the stained specimens gave consistently smaller measurements than living sporozoites, indicating that the prepared specimens may have undergone shrinkage.

In specimens fixed in Zenker's fixative and stained with hematoxylin and eosin (Figure 8), the nucleus stained basophilic and appeared as a well-defined, solid, vesicular ring of chromatin; no nucleolus was observed. Both the anterior and posterior refractile bodies stained lightly eosinophilic. The cytoplasm stained light pink and was relatively homogeneous. Twenty-five sporozoites stained with hematoxylin and eosin averaged 11.6 (9.0 to 13.5) in length. In the region of the anterior refractile body, sporozoites averaged 1.6 (1.0 to 2.5) in width and in the region of the posterior refractile body averaged 1.8 (0.8 to 2.7) in width. Twenty-five flexed sporozoites, stained with hematoxylin and eosin, averaged 6.8 (5.0 to 8.1) in length. In the 25 extended sporozoites, the posterior refractile bodies averaged 4.1 (2.1 to 5.4) in length by 1.8 (1.0 to 2.5) in width and were located an average of 1.2 (0.0 to 3.2) from the posterior end of the sporozoite. The anterior refractile bodies in these same extended specimens, averaged 2.3 (1.5 to 4.3) in length by 1.6 (1.0 to 2.5) in width and were located an average of 2.4 (1.0 to 4.0) from the anterior end of the sporozoite. The nucleus was located approximately 5.2 from the anterior end of the

sporozoite.

Observation of Oocysts

Oocysts were broadly ellipsoid in shape with some being almost spherical. Twenty-five unsporulated oocysts averaged 22.6 (19.9 to 25.9) in length by 20.8 (17.2 to 24.3) in width (Figure 10). The sporoblast was highly granular and was present as a spherical mass which had contracted from the oocyst wall (Figure 10). Fifty sporulated oocysts averaged 21.4 (18.5 to 24.0) in length by 18.7 (17.5 to 24.0) in width (Figure 9) and had a length/width ratio of about 1.1 (1.0 to 1.1). In intact sporulated oocysts, the oocyst wall was about 1.0 thick and appeared as two layers. The outer surface of the oocyst wall was smooth. Micropyle and oocyst residium were not observed; however, 1 to 3 refractile granules resembling polar granules were present in the oocyst fluid (Figure 9). These granules ranged from about 1.0 to 3.0 in length by 1.0 to 1.5 in width and appeared as spherical, bilobed, elliptical or sickle-shaped bodies. Fifty sporocysts averaged 13.1 (12.5 to 15.0) in length by 7.2 (6.5 to 8.0) in width (Figure 9). A cone-shaped Stieda body averaging about 3.0 in length by 1.0 in width was present at one end of the sporocyst. No substieda body was observed. A membrane-bounded sporocyst residium averaging 2.7 in diameter and containing numerous granules averaging about 0.5 in diameter was present within the sporocysts (Figure 16).

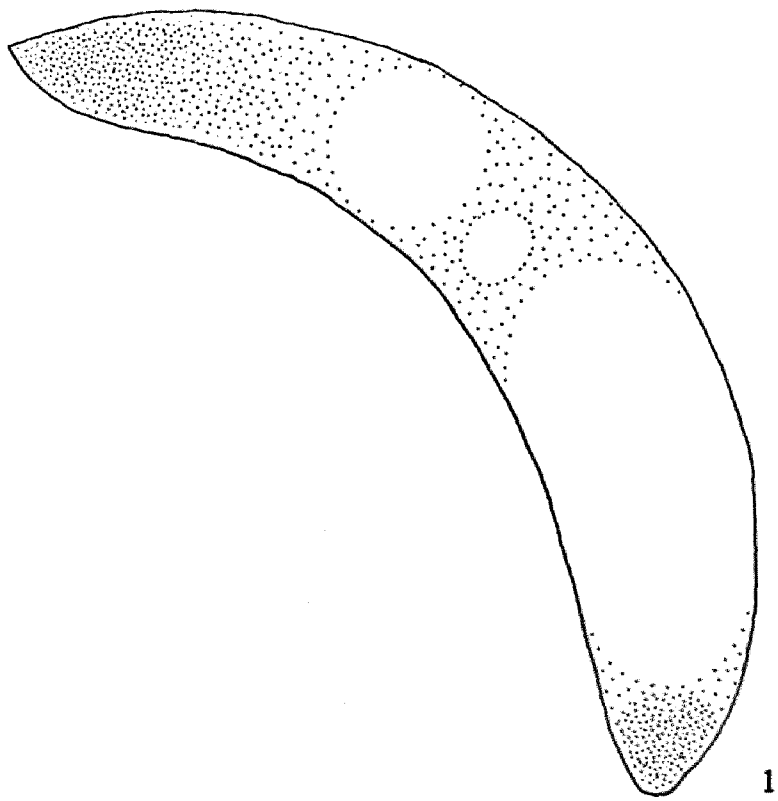


Figure 1. Line diagram of living sporozoite of Eimeria caviae as observed under bright field microscopy. Note nuclear region midway between anterior and posterior refractile bodies, X 12,200.

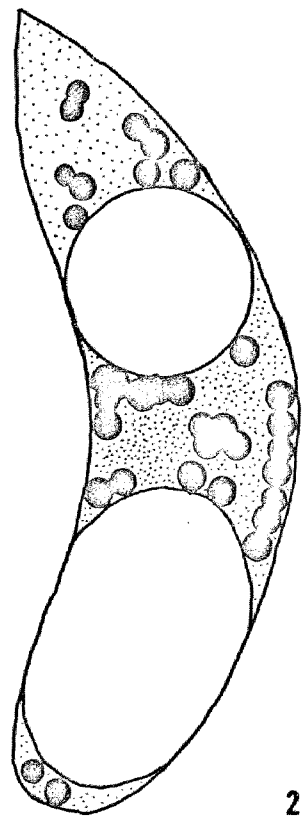


Figure 2. Line diagram of sporozoite of Eimeria caviae fixed in methanol and stained with method of Giemsa. Note distribution of dark granules, X 7,780.

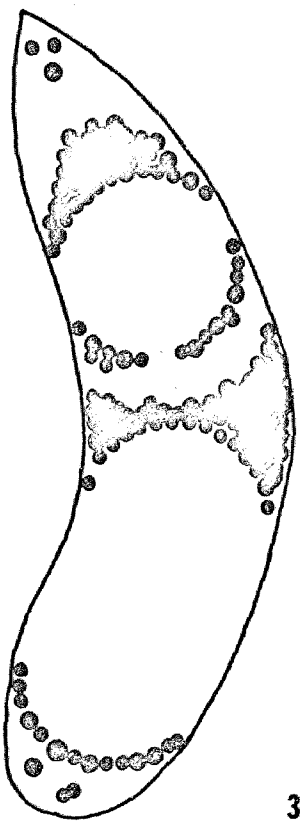


Figure 3. Line diagram of sporozoite of Eimeria caviae fixed in Bouin's fluid and stained with the periodic acid-Schiff (PAS) method. Note distribution of PAS-positive granules, X 10,130.

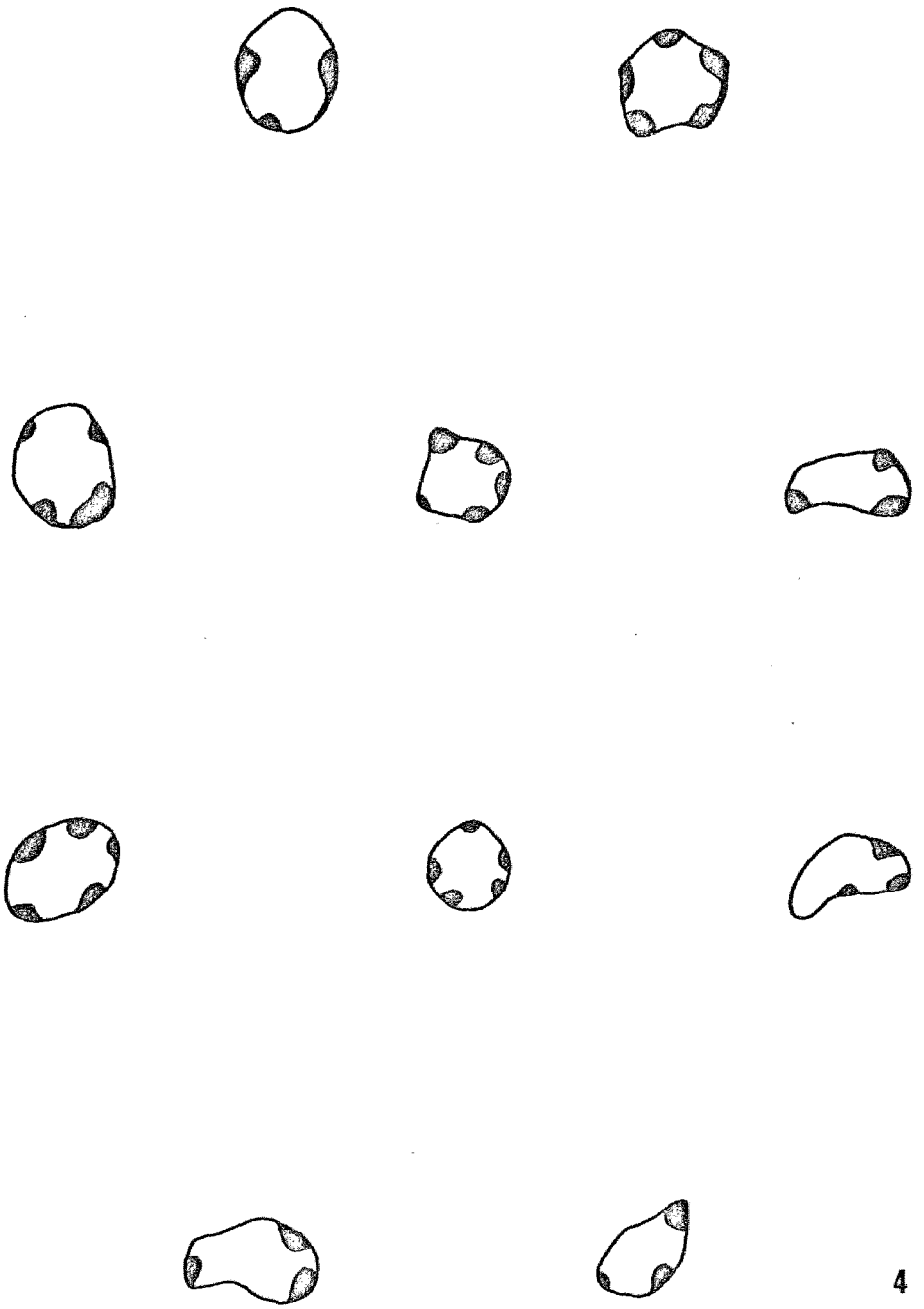
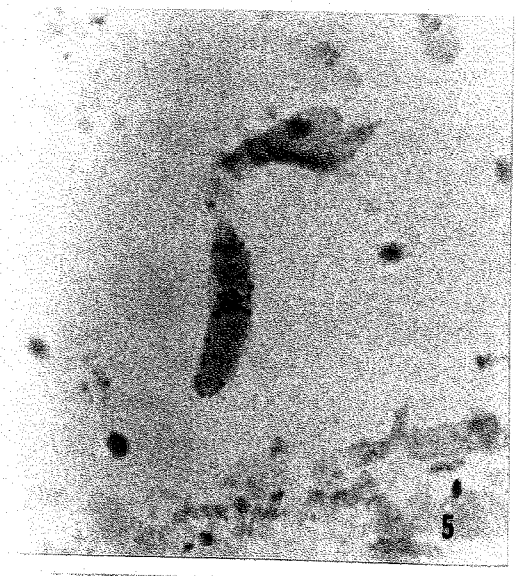


Figure 4. Line diagrams showing variations in the distribution of chromatin in the nuclei of sporozoites of Eimeria caviae stained with the method Fuelgen, X 10,000.

Figures 5-8. Photomicrographs (brightfield) showing sporozoites and oocysts of Eimeria caviae. 5. Giemsa preparation, showing distribution of granules, X 1,800. 6. Periodic acid-Schiff (PAS) preparation showing distribution of PAS-positive granules, X 1,900. 7. Feulgen preparation showing clumps of chromatin in nucleus, X 2,000. 8. Hematoxylin and eosin preparation showing anterior and posterior refractile bodies, X 2,000. 9. Sporulated oocyst of Eimeria caviae, X 1,600. 10. Unsporulated oocyst of Eimeria caviae. Note granular sporoblast, X 1,600.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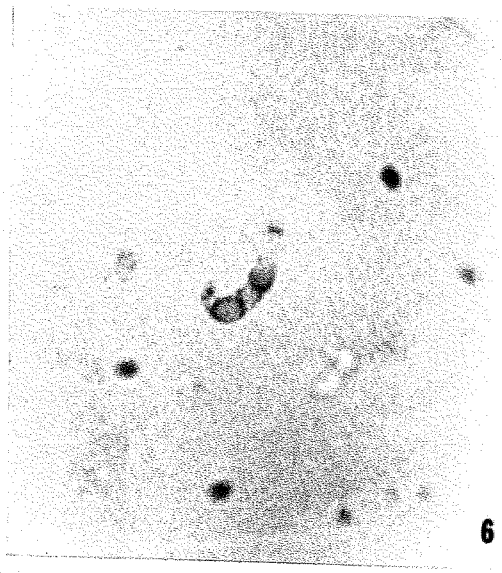
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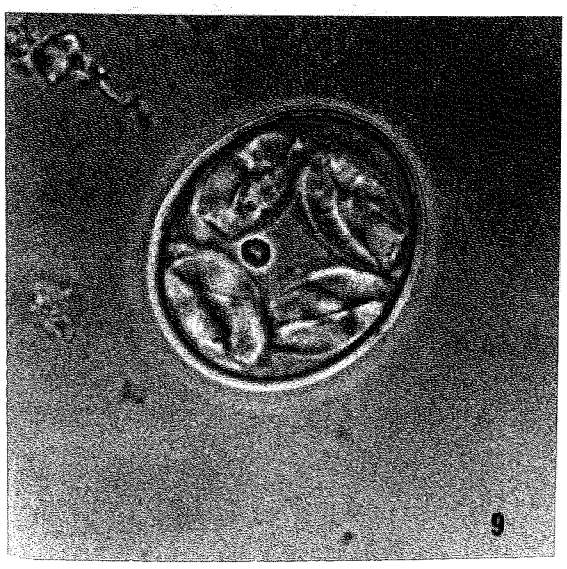
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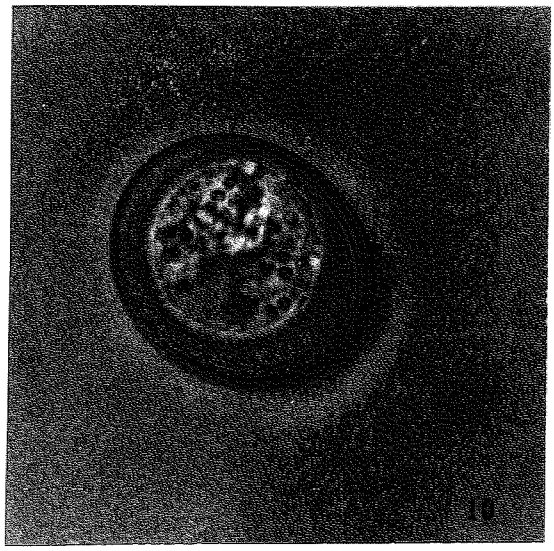
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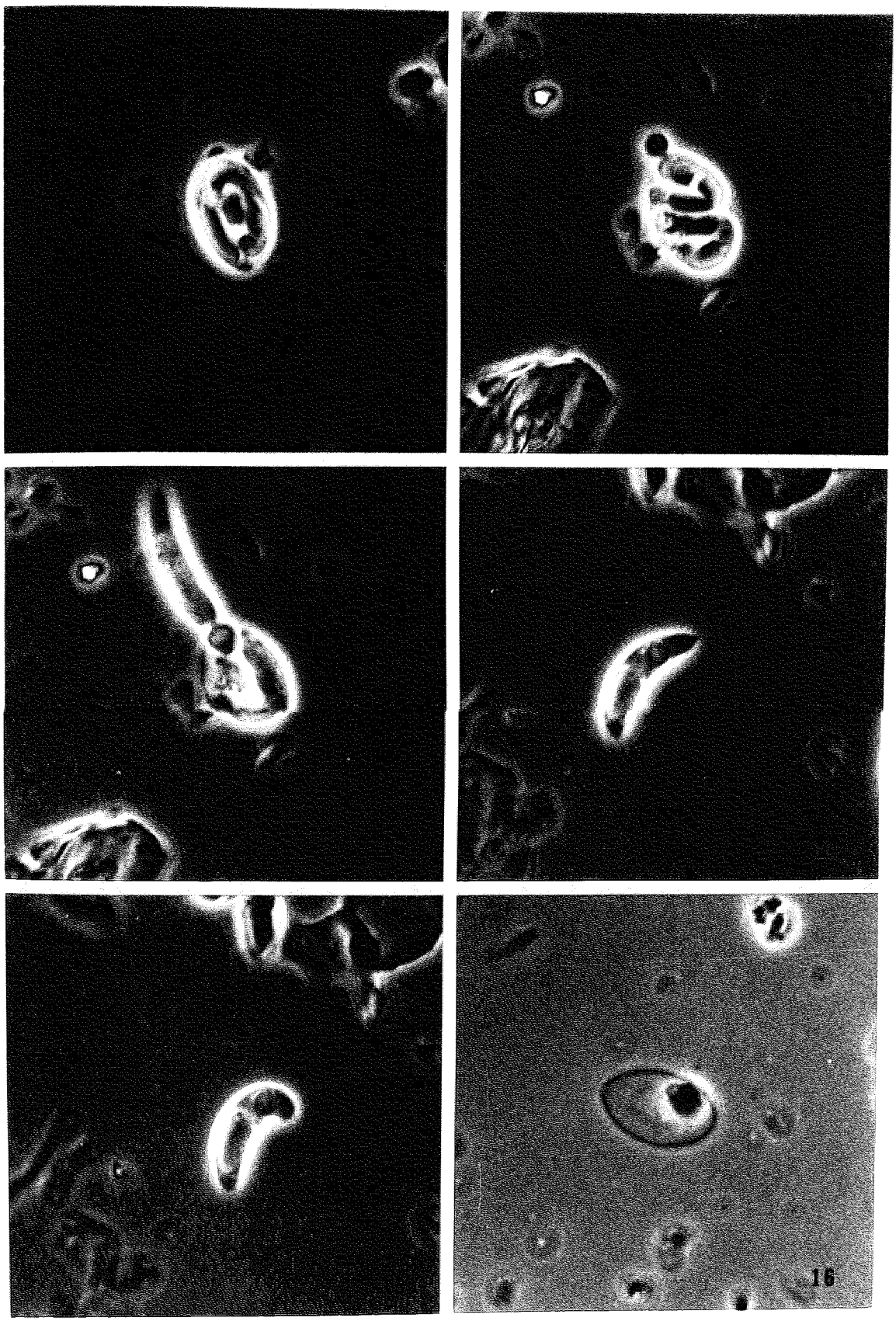
Figures 11-16. Photomicrographs (phase contrast) showing excystation of sporozoites from sporocyst of Eimeria caviae. 11. Sporocyst containing two sporozoites and with Stieda body intact, X 1,500. 12. Sporocyst with sporozoite beginning to emerge, X 1,500. 13. Nearly complete emergence of sporozoite from sporocyst. Note constriction of posterior refractile body, X 1,500. 14. Newly emerged sporozoite in extended position, X 1,500. 15. Newly emerged sporozoite in flexed position, X 1,500. 16. Empty sporocyst containing sporocyst residium, X 1,500.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DISCUSSION

Sporulation and Prepatent and Patent Periods

In the present study, 70% of the oocysts completed sporulation in 9-11 days at room temperature (19-22°C). These data agree most closely with those observed by Lapage (1937) who also found that 60-70% of the oocysts completed sporulation in about 9-11 days. Bugge and Heinke (1921), Sheather (1924) and Henry (1932) showed much shorter sporulation times, e.g., 5-8 days, 5-8 days and 1-3 days respectively; temperature may have been a variable in these latter cases.

The prepatent period of 11-13 days observed in the present study most closely agrees with the 11-14 days observed by Henry (1932). Sheather (1924) recorded a prepatent period of 7-13 days in E. caviae. Lapage (1937) while presenting no data of his own, agrees with the 11-12 day prepatent period noted by Henry (1932).

Clinical Signs

The present study suggests that a dose of 3,000 sporulated oocysts of Eimeria caviae per guinea pig produces little, if any, symptoms. Twenty-four thousand sporulated oocysts per guinea pig, however, seemed to produce slight diarrhea, constipation, crouching and loss of appetite, but did not cause death. The clinical signs which were observed in the present study seem to agree with those of Henry

(1932) but in general seemed less severe. The reason for this may be that Henry inoculated a larger number of oocysts than inoculated in the present study, although Henry did not indicate the number of oocysts inoculated. The most common symptoms of light infection with Eimeria caviae appeared to be mild constipation associated with some irregular stools.

Excystation of Sporozoites

In the present study, it was found that for successful excystation, fresh guinea pig bile was far superior to fresh chicken or mouse bile, as it was also to commercially prepared ox bile. Lotz and Leek (1960) were the first to recognize the importance of using bile in combination with pancreatic enzyme preparations in the in vitro excystation of sheep coccidia. Pratt (1937) failed to obtain excystation of sporozoites from E. tenella oocysts with bile and Itagaki (1954) was unable to obtain excystation from E. tenella sporocysts with sodium taurocholate, but neither investigator used bile or bile salts in combination with enzymes. Doran and Farr (1962) in their study of excystation of oocysts of E. acervulina noted that undiluted chicken bile alone did not excyst or activate sporozoites within free sporocysts. However, a mixture of 2.5% trypsin and undiluted chicken bile added to a suspension of sporocysts gave 85-90% excystation after 1 hour. Jackson

(1962) found that sodium taurocholate and "Tween 80" had an action similar to that of bile in promoting excystation of intact oocysts of sheep coccidia. Also, Doran and Farr (1962) found that various surface active agents such as "Tergitol" and "Microsolv" could be substituted for bile in excystation of free sporocysts of avian coccidia, but the sporozoites died quickly after emerging. Hibbert, Hammond and Simmons (1969), in discussing the effects of pH, buffers, bile and bile acids on excystation, noted that all of the bile acids used in their study gave as good or better results than those of bile.

The swelling and disappearance of the Stieda body during excystation in the present study closely agrees with the observations of other researchers. In E. acervulina (Doran and Farr, 1962), it was noted that the inner portion of the Stieda body became swollen or enlarged before any movement of the sporozoites could be seen. The inner margin of the Stieda body then became less distinct and the body disappeared; at this time the sporozoites began emerging. Swelling and disappearance of the Stieda body was also observed in E. tenella (Nyberg, Bauer, and Knapp, 1968). The mechanism involved in the removal of the Stieda body is still somewhat unclear. However, Doran and Farr (1962) stated that bile salts probably alter the protein or lipoprotein surface of the Stieda body in such a way that it is then readily acted upon by pancreatic enzymes.

Observations of Sporozoites

The morphological characteristics of the sporozoites of Eimeria caviae were similar to those of various Eimeria species previously studied by others (Sheather, 1924; Smetana, 1933; Lapage, 1937; Nyberg and Hammond, 1965; and Hammond et al., 1968).

The most prominent structures observed in the sporozoites of Eimeria caviae were the anterior and posterior refractile bodies. Mazia, Brewer and Alfert (1953) treated sporozoites of an Eimeria species with mercuric bromphenol blue, and demonstrated that the refractile bodies had a great affinity for the stain which indicated their proteinaceous nature. In the present study, refractile bodies were not tested for their proteinaceous properties. Hammond et al. (1968) demonstrated the proteinaceous nature of refractile bodies in Eimeria bovis sporozoites, as did Fayer and Hammond (1969) who studied this species in tissue culture.

The nuclei of the sporozoites of E. caviae were similar in appearance to those species studied by Hammond et al. (1968). The nucleus appeared vesicular and was located between the anterior and posterior refractile bodies. One difference between the nuclei of E. caviae in the present study and those of E. caviae as studied by Lapage (1937) was the presence of a nucleolus in the sporozoites studied by Lapage and an absence of a nucleolus

in the present study. Nyberg and Hammond (1965) noted the presence of a nucleolus in sporozoites of E. bovis as did Roberts, Speer and Hammond (1971) for E. larimenensis from the ground squirrel. Hammond et al. (1968) also noted the presence of a nucleolus in sporozoites of E. bovis, E. auburnensis and an Eimeria species from the Ord Kangaroo Rat. Nyberg and Hammond (1965) did not mention a nucleolus in sporozoites of E. zurnii. Sampson and Hammong (1972) in studying the fine structure of E. alabamensis, noted that sporozoites fixed 5 minutes after inoculation into Madin-Darby bovine kidney cells had no nucleolus, whereas specimens fixed 24 hours later had an enlarged nucleus with a nucleolus. Speer and Hammond (1969) also noted a nucleolus in E. callospermophili from the ground squirrel. Scholtyseck (in Hammond, 1973) is of the opinion that in general, nucleoli are usually present in sporozoites of the genus Eimeria. No satisfactory explanation can be given as to why a nucleolus was not observed in the present study.

A characteristic feature of the nuclei of E. caviae was their peripheral layer of chromatin having 3 to 5 chromatin clumps distributed at irregular intervals along this layer. In this respect, the distribution of chromatin is similar to that observed in sporozoites and merozoites of other species of Eimeria. A peripheral layer of chromatin was observed in merozoites of E. caviae by Lapage (1937). Patillo and Becker (1955) reported chromatin distribution in

E. acervulina sporozoites almost identical to that observed by Lapage (1937). Hammond et al. (1968) also saw small clumps of chromatin occurring at irregular intervals along a peripherally distributed layer of chromatin in sporozoites of E. bovis, E. auburnensis and the Eimeria species from the Ord Kangaroo rat. Ultrastructural studies of sporozoites of Eimeria ellipsoidalis and E. ninakohlyakimovae (Roberts and Hammond, 1970) showed a well-defined peripheral ring of chromatin with some clumping evident. Hammond, Ernst and Goldman (1965) also observed peripheral clumping of chromatin in merozoites of E. bovis which were stained with Feulgen, as did Wacha, Hammond, and Miner (1971), with first generation merozoites of Eimeria ninakohlyakimovae from sheep.

In the present study, in sporozoites stained with periodic acid-Schiff (PAS) numerous PAS-positive granules were concentrated in the middle of the sporozoites, totally obscuring the nucleus. A single layer of these granules was also observed to surround both the anterior and posterior refractile bodies with a few granules scattered in smaller numbers anterior and posterior to the refractile bodies. Gill and Ray (1954), Horton-Smith and Long (1963), Wagner and Foerster (1964) and Ryley (1969) all found PAS-positive granules in sporozoites of E. tenella. According to Ryley (1969), these studies assumed that because magneta staining material was present in granules which could be

destroyed by digestion with amylase, that PAS-positive material was glycogen. Ryley (1969) later pointed out, however, that these PAS reactions are in fact given by any polysaccharide or polysaccharide complex containing adjacent diol groups. An amylase control eliminates substances not having -1, 4-glucoside linkages i.e., dextran, xylan and cellulose; it does not, however, distinguish between glycogen, amylase and amylopectin. In work done with E. tenella, Ryley (1969) is of the opinion that the PAS-positive granules probably contain amylopectin instead of glycogen, and that it occurs in association with protein and functions as an energy reserve similar to amylopectin found in ruman ciliates and in plants.

In sporozoites of E. bovis, Hammond et al. (1968) found that PAS-positive granules were most abundant in the middle region of the body, while some granules were also present in smaller numbers anterior and posterior to the refractile bodies and occasionally lateral to the refractile bodies. However, in sporozoites of the Eimeria species of the Ord Kangaroo rat studied by Hammond et al. (1968) some specimens had no PAS-positive granules while some had granules in small numbers (1 to 5). Roberts, Hammond and Speer (1970) noted that PAS-positive granules at the periphery of the refractile bodies of E. callospermophili were similar to such granules in other Eimeria species, although they were smaller, less numerous, and

less widely distributed than those in E. bovis, E. auburnensis, E. ellipsoidalis, E. ninakohlyakimovae (Roberts and Hammond, 1970) and E. tenella (Ryley, 1969).

In the present study, the nuclei in Giemsa preparations were usually obscured by numerous surrounding granules. Similar granules were noted anterior to the anterior refractile body and posterior to the posterior refractile body. Hammond et al. (1968) reported similar observations in E. bovis sporozoites stained with Giemsa and protargol.

Specimens stained with hematoxylin and eosin showed a basophilic nucleus with a well-defined, solid, vesicular ring of chromatin. This information agrees closely to that of Hammond et al. (1968). The major difference in E. bovis and E. caviae sporozoites stained with hematoxylin and eosin was that Hammond et al. (1968) noted that usually a nucleolus could be seen in E. bovis sporozoites, whereas in the present study, no such nucleolus was noted in E. caviae sporozoites. Refractile bodies of both E. caviae and E. bovis stained eosinophilic and the cytoplasm of both species stained a light pink and appeared homogeneous.

Observation of Oocysts

In this study, the oocysts of Eimeria caviae varied in length from 19.9 to 25.9 and in width from 17.2 to 24.3. This differs somewhat from the size of E. caviae oocysts

given by Henry (1932) in that the oocysts observed by her were smaller than those observed in the present study. The present observations do, however, agree closely with sizes noted for E. caviae oocysts by Bugge and Heinke (1921) and Sheather (1924). In the present study, the oocyst wall measured about 1.0 in thickness and appeared as two layers. Henry (1932) notes the oocyst wall as 0.8 in thickness, appearing as only one layer.

CONCLUSIONS

In the present study, 70% of the Eimeria caviae oocysts completed sporulation in 9 to 11 days at room temperature (19 to 22°C). In 6 guinea pigs studied, the prepatent period of E. caviae was found to range from 11 to 13 days; the patent period ranged from 7 to 13 days.

The first symptom of infection noted in guinea pigs experimentally infected with E. caviae was loss of appetite and constipation. Diarrhea was also noted in three of the six guinea pigs. Extremely severe diarrhea was noted in one guinea pig, which died approximately 5 weeks postinoculation. Apathetic behavior was also a constant symptom, appearing in four of the six guinea pigs.

The results of the present study with E. caviae sporozoites indicate that both bile and trypsin are necessary for excystation of E. caviae oocysts. It was also noted that excystation appeared to begin with swelling of

the Stieda body, which suddenly disappeared. In E. caviae sporocysts freed mechanically from oocysts, the earliest escape of sporozoites was observed 10 to 15 minutes after beginning of incubation at 39°C in a medium consisting of 25% trypsin, 1 ml fresh guinea pig bile and 10 ml Ringer's solution. Once the Stieda body had disappeared, the first sporozoite was free in 10 to 20 seconds.

Living sporozoites observed under bright field and phase-contrast microscopy were found to have a banana-shaped appearance with the anterior end tapering to a point and the posterior end being more rounded and blunt. Freshly excysted sporozoites were motile and exhibited flexion, probing and gliding movements. On a few occasions, rotation of the sporozoite was observed. Fifty of these sporozoites averaged 12.9 (8.1 to 18.9) in length by 2.6 (1.6 to 2.7) in width in the region of the anterior refractile body and 2.8 (2.1 to 3.7) in width in the region of the posterior refractile body. All sporozoites had 1 posterior and 1 anterior refractile body.

Fifty living sporozoites had anterior refractile bodies averaging 2.6 (1.6 to 4.8) in length by 2.6 (1.6 to 3.7) in width and posterior refractile bodies averaging 5.5 (2.7 to 8.1) in length by 2.9 (2.1 to 3.7) in width. These refractile bodies stained eosinophilic in hematoxylin and eosin.

In Giemsa stained smears, 10 to 15 small red

granules were present in the area of the nucleus, while 5 to 12 were present at the anterior end of the sporozoite. These granules were more heavily concentrated around the nucleus and between the posterior refractile body and pellicle. The cytoplasm of these Giemsa stained preparations was homogeneous except at the anterior and posterior ends where it stained a darker blue and near the anterior end, a small, nipple-like structure was present which stained deeply basophilic.

With the periodic-acid Schiff procedure, numerous small PAS-positive granules were concentrated around the nucleus in the center of the sporozoite. A single layer of PAS-positive granules was concentrated around both the anterior and the posterior refractile bodies.

Specimens stained by the method of Feulgen and counterstained with Fast Green showed a thin peripheral layer of chromatin having 3 to 5 chromatin clumps lying at irregular intervals along this layer. No nucleolus was observed.

In specimens stained with hematoxylin and eosin, the nucleus stained basophilic and had a well-defined, solid, vesicular ring of chromatin. No nucleolus was noted.

Twenty-five sporulated oocysts averaged 22.6 (19.9 to 25.9) in length by 20.8 (17.2 to 24.3) in width. Twenty-five sporocysts averaged 13.1 (12.5 to 15.0) in length by 7.2 (6.5 to 8.0) in width. A micropyle and oocyst residium

were not observed; however, 1 to 3 refractile granules resembling polar granules were present. A cone-shaped Stieda body was present at one end of the sporocyst. A substieda body was lacking.

The clinical signs observed in the present study indicate that Eimeria caviae is pathogenic to the host. Also, the present findings concerning sporulation, prepatent and patent periods and clinical symptoms agree closely with those of Henry (1932) in her study of E. caviae. The cytological aspects of the sporozoites of E. caviae in the present study agree closely with those observed for sporozoites of other species of Eimeria as reported by Hammond et al. (1968), and by Nyberg and Hammond (1965).

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